Journal of Tissue Viability (2014) 23, 98–108

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JOURNAL OF tissueviability

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Basic research

Healing efficiency of oligosaccharides generated from almond gum (*Prunus amygdalus*) on dermal wounds of adult rats

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KEYWORDS
Almond gum;
Oligosaccharides;
Arabinogalactan;
Histopathology;
Wound healing

Abstract Almond gum is a naturally occurring polymer produced by almond trees and shrubs. Its abundance, as well as its low cost production makes it a potential feedstock for use in food and pharmaceuticals. In this regard, almond gum oligosaccharides were enzymatically generated, purified and their monosaccharide composition assessed using gas chromatography-flame ionization detector. Oligosaccharide analyses show that the most prominent residues were galactose and arabinose with traces of xylose, rhamnose, glucose and mannose. The glycosyl linkage positions were analyzed using gas chromatography — mass spectrometry

Abbreviations: OAG, oligosaccharide almond gum derivatives; *COAG*, cream formulation supplemented with *OAG*.

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http://dx.doi.org/10.1016/j.jtv.2014.07.001

showing a main chain composed of galactose units [→3)-Gal-(1→] branched mainly with arabinose residues [Ara-(1→]. The potent role of the generated oligosaccharides on rats wound healing was investigated. They have been applied either alone or supplemented, as active substance, with cream formulation, on full-thickness wound created on the dorsum of the rats. The effect of oligosaccharides was assessed by measuring the wound closure percentage, reaching an average of around 100% when applied alone or supplemented to cream formulation. The healing percentage for the control group was only 74.3% at the same day. The histological evaluation of skin sections visualized by light microscopy revealed an improved collagen deposition and an increased fibroblast and vascular densities.

Introduction

Wounds are physical injuries that result in an opening or break of the skin. Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues. Wound healing involves continuous cell—cell and cell—matrix interactions that allow the process in three overlapping phases; inflammation (0—3 days), cellular proliferation (3—12 days) and remodeling (3—6 months) [1]. In fact, platelet aggregation during hemostasis releases a number of soluble mediators starting the healing process [2]. Hemostasis is followed by an early inflammatory phase characterized by vasodilatation, increased capillary permeability, complement activation and polymorphonuclear and macrophage migration into the wound within three days [3].

The availability of drugs able to stimulate the process of wound healing is still limited. Only 1—3% of the drugs listed in western pharmacopoeias are intended to be used on wounds [4]. The use of natural molecules and polymers, as remedies or for tissue engineering, is actually a major approach to repair and/or regenerate tissues [5,6].

Recently, interesting stimulating effects on human skin cell physiology have been shown using plant polysaccharides [7—10]. The strong enhancement of cell viability and proliferation rates in human skin fibroblasts and keratinocytes suggests a positive impact of some carbohydrates on skin regeneration [11]. In fact, it has been reported in previous studies that chitin and chitosan polysaccharides induced the activation of a complement system [12], polymorphonuclear cells [13], fibroblasts and vascular endothelial cells [14]. Additionally, heparin polysaccharides are currently used for the treatment of skin and eye ulcers [15]. Moreover, a family of dextran derivatives and glycosaminoglycans are well known for their comforted therapeutic potential [16,17]. However, regarding the critical uses of these complex polymers in clinical applications due to their structure complexity, the therapeutic fields gave increasing importance to oligosaccharides to replace polymeric structures [18]. In fact, it has been shown that chitosan oligosaccharides could be used as wound dressings [19] and may suppress LPS-induced IL-8 expression in human umbilical vein endothelial cells [20]. Moreover, oligosaccharides of hyaluronic acid increased epidermal cell stemness [21] and promote excisional wound healing through enhanced angiogenesis [22]. It has been reported previously that oligosaccharides generated by enzymatic or chemical hydrolysis of exudate gums, exhibit potent biological activities such as acarbose acting as antidiabetes [23—25], glucose oligomers acting as anticancer drugs [26], and sucralfate acting for epithelial wound healing [27,28].

Despite the huge availability of almond gum in the mediterranean countries (*e.g.* Tunisia), its application on dermatology remains widely ignored. The aim of this work was first, to isolate, purify and characterize oligosaccharides from almond gum (*OAG*), then to investigate their capacity on wound healing, using rats as model. To the best of our knowledge, this work is the first, highlighting the potential role of *OAG* as an agricultural by-product on dermal wound healing.

Materials and methods

Products and chemicals

Almond gum was collected from almond trunks (Achaak variety) in the suburb of Sfax city in Tunisia. Zinc acetate and potassium ferrocyanure were purchased from LOBA Chemie (Mumbai, India). Commercial cream; CICAFLORA®, used as reference, was provided by "Labo MHF" (Mohamed

Hedi El Fekih pharmaceutical industry in Sfax, Tunisia). This cream contains an extract of *Mimosa tenuiflora* obtained from a Mexican tree known for its action on cellular stimulation, promoting the skin reparation [29]. Two creams, provided by the manufacturer "Dahlia Laboratories", were used for the wounds treatment: the first one was a cream formulation, oligosaccharide free (79.7% purified water, 2.3% monopropylene glycol, 0.05% allantoin, 0.4% preservative, 1.5% bee wax, 2.5% caprylic/capric Triglyceride, 4% glyceryl stearate, 1.5% shea butter, 6% stearyl alcohol, 1% isopropyl palmitate, 1% polysorbate 20, 0.05% retinyl palmitate), and the second one was the same cream supplemented with 16% of oligosaccharides from almond gum (*OAG*).

Animals

25 adult rats (*Wistar* variety), weighing 223.96 ± 3.95 g were purchased from the Tunisian Pharmaceutical Industries (SIPHAT, Tunisia). Each animal was kept in an individual cage under standard conditions: 40% humidity, 22 °C temperature and 12-h light—dark cycle. Standard pellet diet and water were provided *ad libitum*. All rats were kept to acclimate for one week before the onset of the experiment. Local Animal Care Committee at Sfax University approved the experimental protocol. All manipulation procedures conducted in this work were in accordance with the International Guidelines for Animal Care [30].

Fungal strain and culture conditions

The enzymatic hydrolysis of almond gum was performed using a mixture of enzymes secreted by *Penicilluim occitanis* Pol6 mutant; provided by Cayla co (Toulouse, France). The Pol6 strain is a hypercellulolytic mutant that was selected after eight rounds of mutagenesis from the CL100 wild type strain [31]. This fungus was cultivated at 30 °C in a modified Mandels liquid medium: KH_2PO_4; 2 g/l, $NaNO_3$; 5 g/l, $MgSO_4$, 7 H_2O; 0.3 g/l, $CaCl_2$; 0.3 g/l, yeast extract; 1 g/l, tween 80; 0.1%, and 2% almond gum powder. The pH value was adjusted to 5.5 with NaOH (1 N) and the medium was supplemented with 1 ml oligoelement solution ($CoCl_2$; 2 g/l, $MnSO_4$ H_2O; 1.6 g/l, $ZnSO_4$ H_2O; 1.4 g/l, and $FeSO_4$ 7 H_2O; 5 g/l) [32,33]. The enzyme production was carried out in a 30-l fermentor (Infors, Suisse) containing almond gum as carbon source. The fermentor was operated at 30 °C, 250 rpm and 1 vvm (volume per volume per minute) aeration. The pH of the medium was maintained at 5.5 using sodium hydroxide (2 N) and orthophosphoric acid (2 M). Antifoam (Strictol 0.1%) was added automatically when required. The fermentor was fed with almond gum (2%) after 3 days of the batch culture. After fourteen days of fermentation, the mixture was centrifuged for 20 min at 7000 rpm. The supernatant was stored at 4 °C and used as the enzyme source. The enzyme activity, performed on the crude enzymatic extract (cellulase, xylanase, mannanase, amylase, pectinase, β-glucanase, β-glucosidase, β-xylosidase), was estimated to 4 U/ml (one unit (U) was defined as the quantity of reducing sugars (μmol) liberated from almond gum per min).

OAG extraction procedure

Almond gum was ground using mortar and pestle and sifted through 0.5 mm sieve. Afterwards, a solution of 1% almond gum prepared in sodium acetate buffer (50 mM, pH 5.5) has been treated for 15 min at 100 °C. The first step of oligosaccharide production was conducted using a 10-l reactor. For this experiment, 2.5 l of almond gum solution (1%) was mixed with 1.5 l of crude enzymatic extract (4 U/ml) obtained by the fermentation of the Pol6 *Penicillium occitanis* fungus using almond gum as substrate. The mixture was adjusted to 10 l with sodium acetate buffer (50 mM, pH 5.5). After the enzymatic hydrolysis of almond gum during 30 min at 50 °C, proteins were removed from the supernatant by adding 1/10 (v/v) zinc acetate (30%) and 2/10 (v/v) potassium ferrocyanure (15%). The resulting solution was centrifuged at 7000 rpm for 20 min. In order to remove the insoluble fraction containing undigested polysaccharides, the supernatant was precipitated with 2 volumes of isopropanol (91%) for 24 h at room temperature. The oligosaccharide fraction dissolved in the supernatant was concentrated by rotary evaporator at 70 °C. The remaining solution was freeze-dried overnight and stored at −20 °C.

OAG purification and structural characterization

In order to further clarify the generated solution, the lyophilized fraction was loaded into a Sephacryl S-200 resin (14 × 1.6 cm) pre-equilibrated with 5 volumes of water. Oligosaccharides were eluted using deionized water at 0.5 ml/min during 2 h. All fractions were collected following the quantification of total sugars as described previously [34], and freeze-dried overnight. The elemental monosaccharide composition (molar ratios) of the

purified oligosaccharides was determined using a modified method of Kamerling et al., [35]. The generated *per*-O-trimethylsilyl methyl glycosides were resuspended in 500 μl dichloromethane, and analyzed by gas chromatography-flame ionization detector (GC-FID). An Agilent GC 6850A instrument equipped with HP-5MS capillary column (30 m length, 0.25 mm diameter and 0.25 μm film thickness) was used. Glycosyl linkage positions of the purified oligosaccharides were determined according to [36] with slight modifications. The generated acetyl compounds were resuspended in 500 μl dichloromethane and analyzed using gas chromatography-mass spectrometry (GC—MS). An Agilent Technologies instrument (GC 6850) equipped with SP-2380 capillary column (30 m length, 0.25 mm diameter and 0.2 μm film thickness) was used for glycosyl residues analysis.

Evaluation of OAG on wound healing

The set of rats was divided into five groups of 5 animals each, as follows: 1st Group: untreated rats served as controls; 2nd Group: rats treated with the commercial cream "CICAFLORA®" used as healing reference; 3rd Group: rats treated with the cream formulation, oligosaccharide free prepared by the manufacturer; 4th Group: rats treated with the same cream formulation supplemented with oligosaccharides (*COAG*) generated in this study by enzymatic hydrolysis of almond gum, and 5th Group: rats treated with the extract of oligosaccharides alone (*OAG*). The comparison of healing activity between the different applied creams was performed after mechanical wounding.

Induction of wounds

All rats were anesthetized before wounding with intramuscular injection of 50 mg/kg ketamine and 5 mg/kg ipnodis. After immobilization, each rat's back was shaved and then a full thickness elliptic excision (1.6 × 1.2 cm) was created (4 mm depth), using a surgical chisel, in the dorsal interscapular region. Thereafter, the animals were individually caged.

Treatment of induced wounds

All wounds were washed daily with saline solution (0.9%) after diethyl ether local anesthesia. Each cream was applied in thin layer (1 g per wound) using a spatula. The treatment took place for 12 consecutive days; the time taken for the complete wound healing of one group over five.

Macroscopic study

The evolution of the wound area was performed daily by drawing its shape with a marker on a transparent paper. The wound area was determined by measuring the weight of the transparent paper fitted to the shape of the wound, normalized as follows:

Area (cm^2) = weight of the transparent paper having the shape of the wound divided by the weight of one cm^2 of the same paper. This method was recently published by Refs. [37,38].

Histopathological evaluation

Histological analysis was performed at the laboratory of Histology and Embryology (Sfax Faculty of Medicine, Tunisia). Biopsies of 6 mm discs were made, including the lesion and the sound skin around the scar as previously described [39]. The excised skin fragments were prepared for examination by light microscopy following the five steps described by Gabe; fixation, circulation, coating, microtomy and coloring [40]. In fact, skin sections were fixed in 10% neutral buffered formalin solution, embedded in paraffin wax, cut into 5 μm thickness and stained with hematoxylin-eosin.

Statistical analyses

All data were evaluated statistically and expressed as mean value ± standard deviation (S.D). In case of multiple comparisons, repeated measurements of Analysis of Variance (ANOVA) were performed in order to compare the mean differences between all healing treatments using Fisher Snedecor test. The average weight of rats, before and after the experiment, was evaluated statistically using the Student's test.

Results

OAG extraction and purification

The oligosaccharides were generated by enzymatic hydrolysis of almond gum following the extraction and purification steps described in the experimental section. The oligosaccharide extraction yield was 33.5%. All fractions collected from gel filtration chromatography after total sugar quantification, showed the presence of 2 peaks (*OAG1* and *OAG2*) represented in Fig. 1. The corresponding

fractions of each peak were pooled, lyophilized, and their monosaccharide composition and structure were determined.

OAG structure identification

Monosaccharide composition of OAG

The monosaccharide composition of *OAG1* and *OAG2*, eluted from the gel filtration chromatography, was determined using GC-FID. Trimethylsilyl monosaccharide derivatives were used as standards. The GC-FID analysis showed that both oligosaccharides *OAG1* and *OAG2* have similar composition (Table 1). In fact, they were mainly composed of galactose and arabinose with traces of xylose, rhamnose, glucose and mannose.

Glycosyl linkage position analysis

The oligosaccharide structure was determined for *OAG1* fraction using GC—MS. Methylation analysis of *OAG1* (Table 2) showed the presence of mono-O-, di-O- and tri-O-substituted residues, indicating a high branching degree. The presence of the major units →3)-Gal-(1→ and Ara-(1→ confirm the arabinogalactan structure of *OAG*. Terminal xylosyl and 3,4-linked rhamnosyl revealed branch points and/or substitutions with non-osidic compounds. Once the *OAG* structure identified, their dermal application on rat wounds was investigated.

Body weights evolution of rats

The effect of *OAG1* and *OAG2* pooled together was tested among other creams on rat wounds for 12 days. The average body weight of each group listed in Table 3 showed no significant differences ($p < 0.05$) between the treated groups and the control.

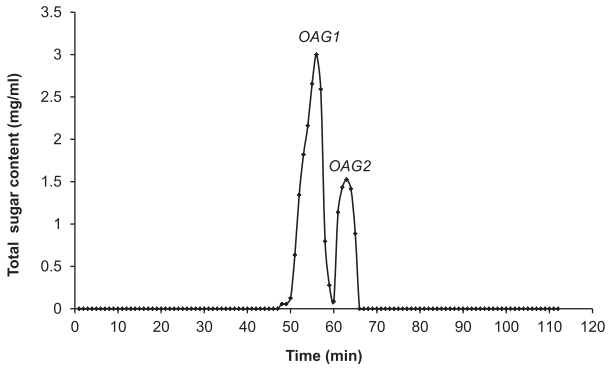

Fig. 1 Total sugar quantification after *OAG* purification on Sephacryl S-200 filtration gel.

Table 1 *OAG1* and *OAG2* monosaccharide composition (molar ratios), assessed by GC-FID using trimethylsilyl monosaccharide derivatives as standards.

Compound	*OAG1*	*OAG2*
Arabinose	1	1
Galactose	0.52	0.51
Xylose	0.093	0.09
Rhamnose	0.08	0.1
Glucose	0.08	0.1
Mannose	0.05	0.05

Macroscopic study of wounds

The surface of the wound area was monitored during 12 days to assess the wound healing potential of *OAG*. Fig. 2 showed the wound pictures taken on the 1st, 3rd, 8th, 10th and 12th day corresponding to the induction of the wounding on the 1st day, the end of the inflammatory phase on the 3rd day, the formation of granulation tissue on the 8th day and the re-epithelialization phase on the 10th and 12th days. Interestingly, the obtained results showed that at the 10th day; the 2nd, 4th and 5th groups showed significant and advanced healing behavior. Moreover, the 4th and 5th groups presented an active *in vivo* wound healing and showed a complete contraction on the 12th day compared to the reference group. Delayed wound healing process was observed for the control and the cream formulation oligosaccharide free groups (1st and 3rd group, respectively) (Fig. 2).

Statistical analyses of the wound healing effect

The evolution of the wound healing percentage was illustrated in Fig. 3 and Table 4. The healing process had increased steadily over time in all the tested samples. To better assess the effectiveness of the different treatments, the healing percentages were compared on the 8th and 12th days (Table 4). The obtained results showed that the treatments of the 2nd, 4th and 5th groups were the most efficient with the following healing percentages [77.71 and 98.98]; [78.38 and 100] and [71.48 and 98.98], at the 8th and 12th day, respectively. These percentages were significantly higher than those found for the 1st and 3rd groups; having the following healing percentages [50.13 and 74.38] and [60.33 and 80.85], at the 8th and 12th day, respectively (Fig. 3). In order to record the significant differences between all treatments, a Fisher Snedecor test was performed between the different groups (Table 4). The obtained results

Table 2 Glycosyl linkage position analysis of *OAG* determined by GC—MS after methylation, hydrolysis, reduction and acetylation. Gal; galactosyl, Ara; arabinosyl, Xyl; Xylosyl, Rha; rhamnosyl.

Monosaccharide	Derivative	Retention time (min)	Glycosyl linkage
Galactose	1,3,5-tri-O-acetyl-1-deuterio-2,4,6-tri-O-methyl D-galactitol	9.9	→3)-Gal-(1→
Arabinose	1,5-di-O-acetyl-1-deuterio-2,3,4-tri-O-methyl D-arabinitol	11.4	Ara-(1→
Xylose	1,5-di-O-acetyl-1-deuterio-2,3,4-tri-O-methyl D-xylitol	11.8	Xyl-(1→
Rhamnose	1,3,4,5-tetra-O-acetyl-1-deuterio-2,6-di-O-methyl D-rhamnitol	14.9	→3,4)-Rha-(1→
Galactose	1,5,6-tri-O-acetyl-1-deuterio-2,3,4-tri-O-methyl D-galactitol	25.8	→6)-Gal-(1→

showed that the healing effect of oligosaccharides applied alone (*OAG*) or supplemented to cream formulation (*COAG*) were significantly different from the control group at 99% confidence. Moreover, no significant differences were observed between the *OAG* and *COAG* treatments compared to the reference cream (Table 4).

Histological evaluation of the healing behavior

On the 12th day of the treatment, all rats were sacrificed and skin sample discs were taken at the scar. The analysis of the histological sections showed that the epithelial regeneration was incomplete for the 1st and 3rd groups (Fig. 4a and c, respectively). Nevertheless, a complete epithelial regeneration with a thick and well-structured epidermis was observed in biopsies taken from rats of the 2nd, 4th and 5th groups (Fig. 4b, d and e, respectively), compared to the 1st and 3rd groups, which were unable to reform the epidermis.

Granulation tissue surmounted by fibrino-leucocytic magma was noticed on rats treated with the cream formulation, oligosaccharide free (3rd group), indicating thereby the presence of an ulcerated zone. However, the formulation supplemented with oligosaccharides (4th group) lead to the complete wound healing without any side effect.

Discussion

OAG generated by enzymatic hydrolysis of almond gum is mainly composed of galactose and arabinose. This result support previous works [41,42] demonstrating the classification of almond gum as an arabinogalactan polymer. This composition was very similar to other exudate gums such as those extracted from peach tree (*Prunus persica*) [43], *Acacia senegal* [44], nectarine gum [45] or *Acacia mearnsii* gum [46].

The examination of body weights during the experimental period suggested that all groups were uniform and indicates the absence of any side effect of the tested products on the growth rate of rats.

The macroscopic study showed that during the first three days of the treatment, all the wounds present similar appearance. This period was marked by a brown blood clot deposition. The coagulated dermal desiccation, turned into a scar, was formed by a necrotic tissue remnants used to protect the surface from any external irritation. Under this scar, the granulation tissues started to spread, divided cells from the epidermal wound borders proliferate and contribute thereby to faster differentiation and wound healing. According to Lawrence, this process occurs after 21 days without any treatment [47]. An accentuated inflammatory rim appeared around the wounds created for these groups. Nevertheless, no inflammation has been

Table 3 Average of the body weights for the different groups of rats before and after treatment.

Average body weights (g) ± S.D.	Group 1	Group 2	Group 3	Group 4	Group 5
Before treatment	223.8 ± 4.03	224.4 ± 3.62	223.6 ± 3.97	224 ± 4.80	224 ± 3.32
After treatment	230.2 ± 4.25	231.43 ± 3.38	230 ± 4.32	230.59 ± 4.12	231.32 ± 3.61

Notes. Group 1; rats servingas control, Group 2; rats treated with a healing reference «CICAFLORA®», Group 3; rats treated with the classical formulation prepared by a pharmacist oligosaccharides free, Group 4; rats treated with the classical formulation supplemented with oligosaccharides (COAG) and Group 5; rats treated with the extract of oligosaccharides alone (OAG).S.D. denotes the standard deviation calculated from the five measurements of each group.

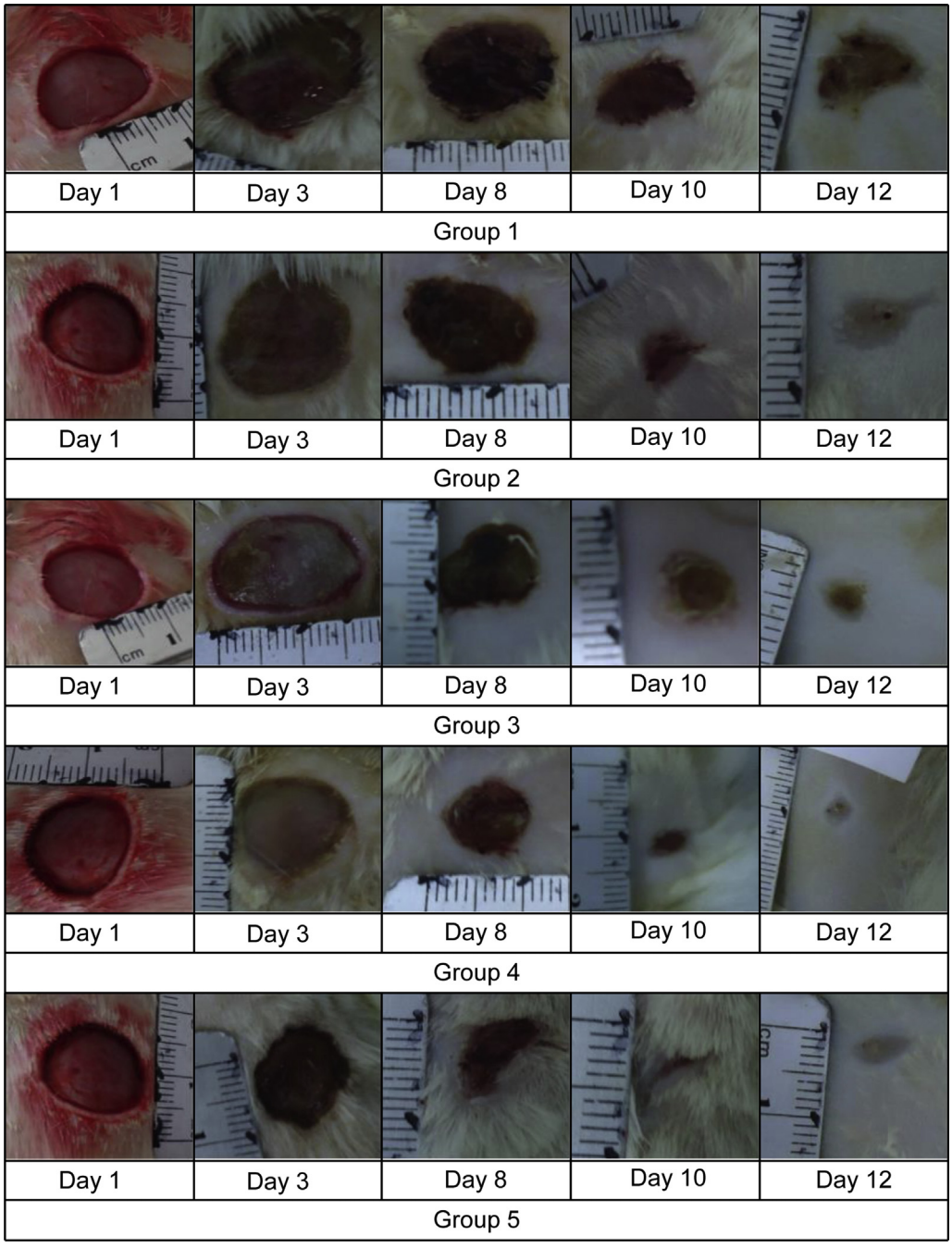

Fig. 2 Pictures of the wounds healing taken for the different groups on the 1st, 3rd, 8th, 10th, and 12th day. Group 1: control group. Group 2: reference group. Group 3: cream formulation oligosaccharides free. Group 4: cream formulation supplemented with oligosaccharides (*COAG*). Group 5: oligosaccharides alone (*OAG*).

recorded for the remaining groups. This finding could be attributed to the anti-inflammatory and antibacterial properties of *OAG* and the reference cream as previously described for other oligosaccharides [29,48].

The statistical analyses show that the healing process was improved in the presence of oligosaccharides. In fact, a wound healing average of 50% was observed at the 5th day for the 5th group treated with *OAG*. However, this state was reached one day after, for the 2nd and 4th groups treated with the reference cream and the *COAG*, respectively. Delayed healing period was observed for the 3rd and 1st groups (7th and 8th day of the treatment, respectively, to reach 50% of the healing time). The healing effect of the cream formulation,

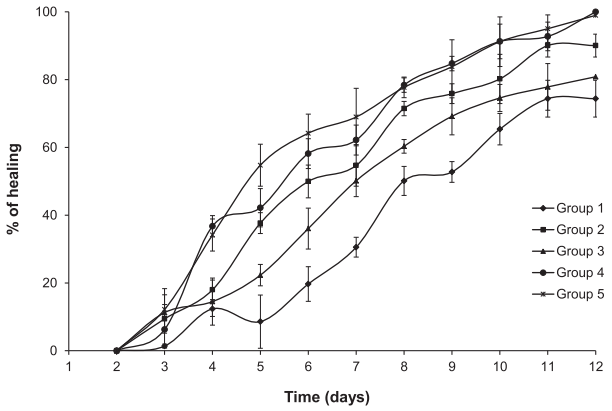

Fig. 3 Daily evolution of the average percentages of wound healing during 12 days: Group 1; control group, Group 2; reference group, Group 3; rats treated with the cream formulation oligosaccharide free, Group 4; rats treated with the cream formulation supplemented with oligosaccharides (*COAG*), Group 5; rats treated with oligosaccharides only (*OAG*). The standard deviations were calculated from the five measurements of each group.

oligosaccharide free, was not significantly different from the control group. Therefore, the healing effect of *COAG* was improved after the incorporation of oligosaccharides.

The histological study of the skin discs excised from rats treated either with *OAG* or with *COAG* showed low collagen density, this behavior was associated with significant vascularization and optimal epithelialization, compared to the other rat groups. *OAG*, supplemented to the formula or applied alone, was more efficient for the tissue regeneration involved in the healing process. In fact, in normal tissues; strength, integrity and structure are provided by collagen. When collagen is deposited with high amount in the wound site, the current anatomy of the structure is lost and a fibrosis occurs. The observation for the granulation tissues were concordant with those performed by Tsala and co-workers; where they explained the shortness of the epithelialization time by the anti-inflammatory effect of the plant extract during the wound repair [2]. The histological study was concordant with previous works. In fact, if collagen is deposited in low amount, the wound may still weak and dehisce [49]. Other findings from Buffoni and co-workers have demonstrated that the healing is not complete until the disrupted surfaces are firmly knit by collagen [50]. Moreover, the healing requires a contribution of several tissues and cell lineages [51]. In fact, this process involves blood clotting, formation of fibrin, inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. The basic principle of optimal wound healing, in order to minimize skin damage, is to provide adequate tissue perfusion and oxygenation, appropriate nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part [52]. Similar effect has been observed by Gao and co-workers; where the oligosaccharides generated from the partial hydrolysis of hyaluronic acid, promote the wound healing and amplify the production of granulated tissue, collagen deposition and fibroblast proliferation [22]. This may explain the effect of *OAG* on wound healing by improving the hematopoietic system

Table 4 Comparison of the average percentages of wound healing between the five groups on the 8th and the 12th days of treatment.

Compared groups	Experimental value of Fisher (F_0)		Critical value of Fisher (F_c)		Level of significance	
	8th day	12th day	8th day	12th day	8th day	12th day
Group 1 and Group 2	20.5	6.04	11.26	5.32	**	*
Group 1 and Group 3	4.66	0.98	5.32	5.32	N.S	N.S
Group 1 and Group 4	34.85	17.26	11.26	11.26	**	**
Group 1 and Group 5	27.57	16.58	11.2	11.26	**	**
Group 2 and Group 3	7.32	6.27	5.32	5.32	*	*
Group 2 and Group 4	5.28	4.96	5.32	5.32	N.S	N.S
Group 2 and Group 5	2.83	4.3	5.32	5.32	N.S	N.S
Group 3 and Group 4	37.67	30.4	11.26	11.26	**	**
Group 3 and Group 5	11.34	30.99	11.26	11.26	**	**
Group 4 and Group 5	0.032	0.066	5.32	5.32	N.S	N.S

Notes. Group 1; Rats served as control, Group 2; Reference rats treated with «CICAFLORA®» cream, Group 3; Rats treated with the classical formulation oligosaccharides free, Group 4; Rats treated with the classical formulation supplemented with oligosaccharides (COAG) and Group 5; Rats treated with the extract of oligosaccharides alone (OAG).N.S: Not Significant difference, * and ** denote significant differences at 95% and 99% of confidence, respectively.

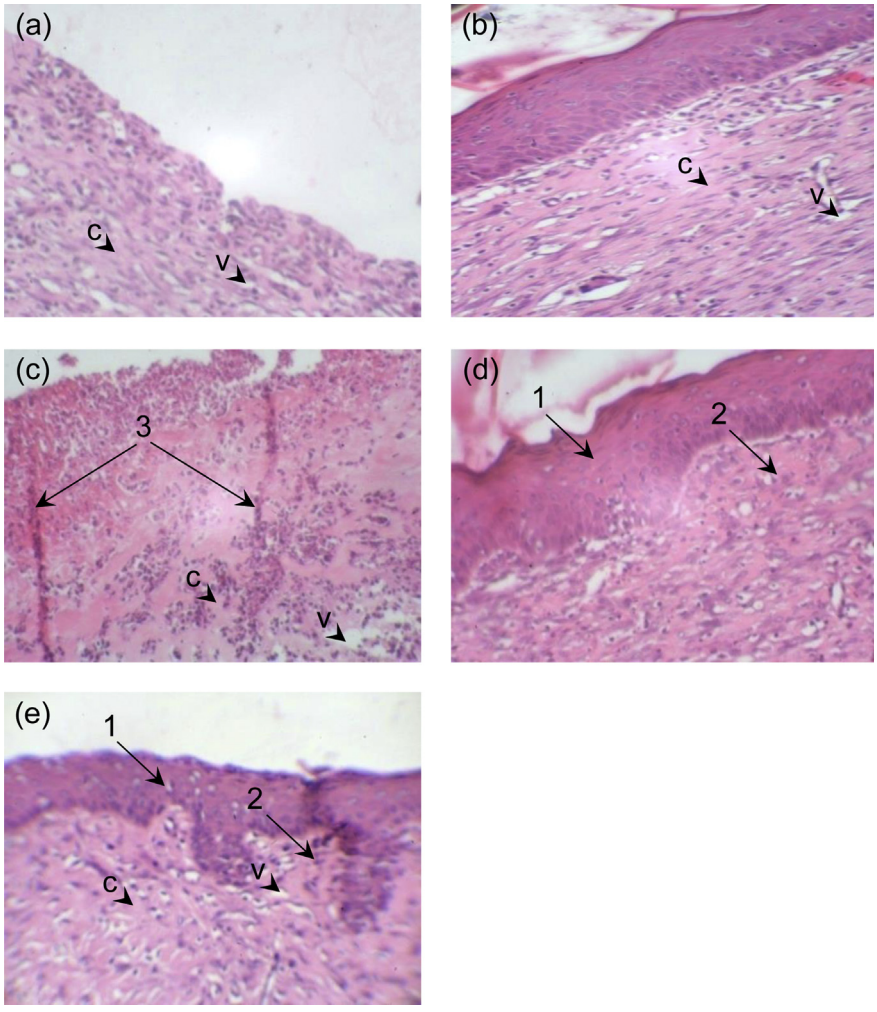

Fig. 4 Histological sections of a scarred area. (a); Untreated rats, (b); Rats of reference group, (c); Rats treated with the cream formulation oligosaccharides free, (d); Rats treated with the cream formulation supplemented with oligosaccharides, (e); Rats treated with oligosaccharides. Tissues were stained with hematoxylin-eosin and visualized 100 folds using light microscopy. All scale bars are equal to 100 μm. 1; epidermis, 2; dermis, 3; ulcerated area, c; collagen, v; blood vessels.

involving the early closure of the wound as previously reported [53].

Conclusion

The results of this study indicate the potential role of oligosaccharides, generated by enzymatic hydrolysis of almond gum, on wound healing. The oligosaccharides applied alone or supplemented to cream formulation showed an acceleration of wound healing, compared to the control group. This result may be attributed to the promotion of neo-blood vessels and collagen formation by *OAG*. Finally, this study provides interesting data regarding the wounds treatment and paves the way toward clinical trials of almond gum oligosaccharides in human.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

We express our gratitude to Prof. Gérard Tiraby and Dr. Henry Durand (Cayla Company, France) for kindly supplying the *Penicillium occitanis* (Pol6) strain used in this work. We thank Dr. Tarak Rebaï, head of the Histology Laboratory, Sfax Medicine Faculty, Tunisia, for his pertinent interpretations on the histological study. We extend our thanks to Pr. Jamil Jaoua, founder and former head of the English Unit at the Sfax Faculty of Science, for having proofread this paper. This work was funded

by the Ministry of Higher Education, Scientific Research and Technology in Tunisia.

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